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Gramicidin, Valinomycin, and Cation Permeability of *Streptococcus faecalis*

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Gramicidin and valinomycin in concentrations of 10^{-7} and 10^{-6} M, respectively, inhibited the growth of *Streptococcus faecalis*. Inhibition of growth was associated with loss of Rb^+ and K^+ from the cells, and could be reversed by addition of excess K^+. Cells treated with these antibiotics exhibited greatly increased permeability to certain cations; no effect was observed on the penetration of other small molecules. Unlike normal cells, cells treated with gramicidin rapidly lost internal Rb^+ by passive exchange with external cations, including H^+, all monovalent alkali metals, NH_4^+, Mg^{++}, and tris(hydroxymethyl)aminomethane. Exchange was rapid even at 0 C and was independent of energy metabolism. The effect of valinomycin was more selective. Cellular Rb^+ was rapidly displaced by external H^+, K^+, Rb^+, and Cs^+; other cations were less effective. The exchange was independent of metabolism but strongly affected by temperature. Under certain conditions, polyvalent cations inhibited exchange between ^{86}Rb and Rb^+ induced by valinomycin. The antibiotic apparently neither stimulates nor inhibits the energy-dependent K^+ pump of *S. faecalis*, but exerts its effect on the passive permeability of the membrane to cations. The increased permeability to specific cations induced by gramicidin and valinomycin is a sufficient explanation for the inhibition of growth, glycolysis, and other processes.

In 1944, Hotchkiss (12) reviewed what was known at the time concerning the mechanism of action of the related antibiotics tyrocidin and gramicidin (GMCD). He concluded that tyrocidin grossly disrupts the cytoplasmic membrane of bacteria by a process analogous to that caused by cationic detergents (17), and this interpretation was confirmed by more recent work (14). GMCD appeared to act at a different level, inhibiting phosphorylation reactions by a mechanism involving K^+. Subsequently it became apparent that GMCD also acts upon membranous structures; it is a powerful uncoupling agent for oxidative phosphorylation in bacteria and mitochondria (6, 18). The structure of GMCD, a mixture of closely related linear polypeptides, has been established (21). Very recently, evidence was obtained that the primary action of GMCD may be to increase the permeability of the mitochondrial membrane to cations; the uncoupling of oxidative phosphorylation appears to be a secondary consequence of this alteration in membrane structure (3).

The cyclic polypeptide antibiotic valinomycin (22; VAL) also uncouples oxidative phosphorylation under certain conditions (11, 20). The primary effect of VAL, like that of GMCD, appears to be on cation transport. Addition of VAL to respiring mitochondria stimulates K^+ uptake and ejection of H^+. It is believed that this antibiotic increases the permeability of the mitochondria to certain cations, and that it may also react directly with a specific K^+ carrier (10, 11 19, 20). Virtually nothing is known concerning the effect of VAL on microorganisms.

The present experiments were initiated as part of a program of research on the transport of K^+ in *Streptococcus faecalis*. This microaerophilic organism derives energy entirely by fermentation of glucose to lactic acid; the absence of oxidative phosphorylation considerably simplifies the interpretation of our findings. We shall present evidence that the inhibition of the growth of *S. faecalis* by GMCD and VAL ultimately results from potassium deficiency. Both antibiotics interact with the cytoplasmic membrane to increase its permeability to cations, and thus interfere with the selective accumulation of K^+.

MATERIALS AND METHODS

The procedures used in the present study have been described in earlier papers (9, 9*a*), and a brief summary will suffice here.

Growth media. S. faecalis strain 9790 was usually grown on defined media; both K^+ and Rb^+ support growth (9*a*, 15). Because of the technical convenience of ^{86}Rb as an isotope of Rb^+, cells grown on Rb^+ were used whenever possible. Glucose served as energy source, and growth was followed by turbidimetry at 600 mμ.

(i) Medium NaM is buffered with sodium maleate (200 to 350 mM Na^+) and contains various amounts of K^+ or Rb^+ (9*a*).

(ii) Medium KM is buffered with potassium maleate. It contains 200 mM K^+ but no Na^+.

(iii) Medium TEA is buffered with triethanolamine (15) and is supplemented with K^+ or Rb^+.

(iv) Medium NaTY is a complex medium (9*a*, 26) containing 150 mM Na^+ and 5 mM K^+.

Cation content. Cells were loaded with ^{86}Rb by growth or incubation for several hours in media containing ^{86}Rb of known specific activity. Under these conditions, the cells exchange internal Rb^+ for ^{86}Rb (9*a*); at equilibrium the radioactivity of the cells is a measure of their Rb^+ content. Samples were harvested by filtration on membrane filters (Millipore Corp., Bedford, Mass.), washed with water or 2 mM $MgCl_2$, and counted. When desired, K^+, Na^+, and Rb^+ were extracted and estimated by flame photometry.

Other procedures. Protoplasts were prepared by incubation with lysozyme as described by Abrams (1). Glycolysis was monitored at constant *p*H by automatic titration of the lactic acid produced, with a Radiometer *p*H-stat. In a few experiments, uptake of H^+ by the cells was likewise followed by automatic addition of standardized HCl at constant *p*H.

Antibiotics. Gramicidin and tyrocidin were commercial products (Nutritional Biochemicals Corp., Cleveland, Ohio). Valinomycin was a gift from J. C. MacDonald. Antibiotics were dissolved in ethyl alcohol; control flasks always received a volume of ethyl alcohol equal to that of the experimental flasks, usually 1% by volume.

RESULTS

Inhibition of growth. Cells growing in medium KM, containing K^+ as major cation, were little affected by addition of either VAL (5 μg/ml, 5×10^{-6} M) or GMCD (2 μg/ml, 1×10^{-6} M), though higher concentrations of GMCD did inhibit growth. However, in media containing predominantly Na^+ or triethanolamine, addition of VAL at 1 μg/ml (10^{-6} M) reduced the growth rate by 80%; GMCD at 0.2 μg/ml (10^{-7} M) stopped growth completely. In both cases, addition of excess K^+ permitted resumption of growth. Representative experiments of this type are shown in Fig. 1.

Cells exposed to GMCD formed the expected number of colonies when plated on KM medium,

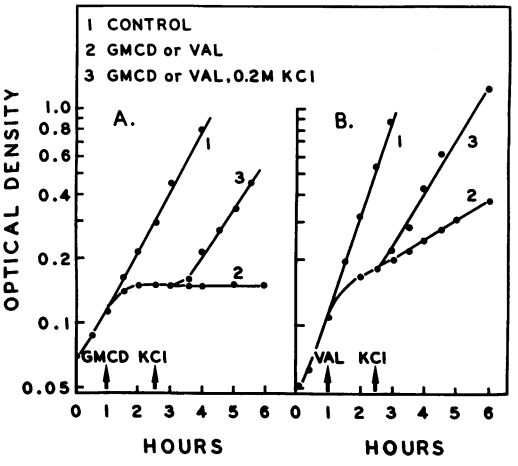

FIG. 1. *Effect of gramicidin and valinomycin on growth of Streptococcus faecalis. (A) An exponentially growing culture in medium TEA was divided (arrow) and the following additions were made: 1) control, ethyl alcohol only; 2) gramicidin (GMCD), 0.2 μg/ml; 3) gramicidin, followed by 0.2 M KCl at 2.5 hr. (B) A parallel experiment in medium NaM, with additions as follows: 1) Control; 2) valinomycin (VAL), 1 μg/ml; 3) valinomycin, followed by 0.2 M KCl at 2.5 hr.*

but only 0.1% were viable on medium NaM. It appears that GMCD is not readily dissociated from the cells by dilution. Cells treated with VAL formed the same number of colonies on both media. Gross lysis was never observed with either antibiotic; this contrasts with the effects of tyrocidin which stopped growth at 1 μg/ml (0.8×10^{-6} M) in all the media tested and also induced visible lysis.

Experiments with *S. faecalis* growing in medium NaM-^{86}Rb provided direct evidence for an effect of VAL and GMCD on the metabolism of inorganic cations. During the exponential phase of growth, untreated cells contained some 700 μmoles of ^{86}Rb per g (dry weight) of cells. Within 10 min, the ^{86}Rb content fell to 300 μmoles/g upon addition of VAL (1 μg/ml) and to 30 μmoles/g with GMCD (0.2 μg/ml). These results suggest the interpretation to be supported below, that both antibiotics interfere with the selective accumulation of K^+ and Rb^+; growth of the cells then becomes dependent upon high extracellular concentrations of these cations.

Retention of K^+ and Rb^+ During exponential growth, *S. faecalis* contains up to 800 μmoles of K^+ or Rb^+ per g of cells, together with small amounts (50 to 150 μmoles/g of cells) of Na^+. In a series of measurements, the ratio of total monovalent cations to total phosphorus was 1.0 to 1.3, suggesting that a large fraction of the cations may be associated with cellular phosphorus compounds.

Cells loaded with K^+ or ^{86}Rb may be washed repeatedly with water, KCl, or NaCl (0.1 M) without appreciable loss of ^{86}Rb. However, cells treated with GMCD or VAL lost ^{86}Rb to varying degrees upon washing with dilute solutions of salts (Table 1). In cells treated with GMCD, ^{86}Rb was lost upon washing with all the cations tested. The effect of VAL was more selective. ^{86}Rb was displaced much more rapidly by K^+, Rb^+, and Cs^+ than by the other cations. The displacement of ^{86}Rb is due to exchange with cations from the wash fluid, as will be shown below. Entirely analogous results were obtained with protoplasts, pointing to the cytoplasmic membrane as the target organelle of these antibiotics.

It was shown above that high concentrations of K^+ nullify the inhibition of growth by both VAL and GMCD (Fig. 1). Two lines of evidence support the conclusion that 0.2 M K^+ does not interfere with the action of the antibiotics per se, but merely makes good the loss of K^+ from the cells. Cells were loaded with ^{86}Rb and suspended in 0.2 M KCl. Upon addition of the antibiotics (1 μg/ml), the ^{86}Rb was instantaneously displaced. *S. faecalis* was grown in medium TEA supplemented with 0.1 M ^{86}Rb. Addition of VAL or GMCD had little effect on growth. However, cells harvested from such cultures lost ^{86}Rb upon washing with salts (see Table 1), whereas normal cells retained ^{86}Rb

TABLE 1. *Release of ^{86}Rb by Streptococcus faecalis treated with valinomycin or gramicidin*[a]

Wash	Percentage of ^{86}Rb retained		
	Ethyl alcohol only	Valinomycin	Gramicidin
Water	100	87	59
LiCl, 20 mM	100	73	0.5
NaCl, 20 mM	94	62	1
KCl, 20 mM	95	9	0.5
RbCl, 20 mM	97	11	0.5
CsCl, 20 mM	90	13	1.5
NH_4Cl, 20 mM	95	61	0.5
$MgCl_2$, 20 mM	98	67	4.5
Tris chloride, 20 mM	95	77	22

[a] *S. faecalis* was loaded with ^{86}Rb as described in the text. The cells were washed and resuspended in water at 0.5 mg/ml. To portions of this suspension were added valinomycin, gramicidin (1 μg/ml final concentration), or ethyl alcohol alone (1% final). After 5 min at room temperature, 1-ml samples were filtered and washed on the filter three times, for 1 min at a time, with 2 ml of the various salt solutions. The original cells washed with water contained 6,100 counts per min per mg of dry cells. This is taken as 100%, and retention of Rb ^{86}Rb is expressed as the percentage of ^{86}Rb remaining in the cells after exposure to ethyl alcohol or antibiotics and washing.

Cation retention by *S. faecalis* was exquisitely sensitive to GMCD and VAL. Exposure of cells under the conditions shown in Table 1 to as little as 0.05 μg of GMCD per ml (2×10^{-8} M) or 0.2 μg of VAL per ml (2×10^{-7} M) resulted in significant loss of ^{86}Rb. By contrast, the following agents were without effect: ethyl alcohol, 0.25 M; *n*-butanol, 0.15 M; polymyxin B, 25 μg/ml; streptomycin, 10^{-3} M; Triton x-100, 10 μg/ml; cetyltrimethylammonium bromide, 2×10^{-5} M; dodecyltrimethylammonium bromide, 3×10^{-5} M; sodium lauryl sulfate, 3×10^{-5} M; *n*-dodecylamine, 5×10^{-5} M. The four ionic detergents caused lysis of growing cells at concentrations above 1×10^{-4} M.

Other effects on membrane permeability. To assess the effects of VAL and GMCD on the retention of other compounds, cells were loaded with ^{32}P-orthophosphate (Pi), ^{14}C-alanine, or ^{14}C-glutamate by incubation in the presence of glucose. Chloramphenicol (50 μg/ml) was added to prevent incorporation of ^{14}C-amino acids into proteins. The cells were washed, suspended in water, and exposed at room temperature to VAL or GMCD (1 μg/ml). The labeled compounds were not displaced upon subsequent washing of the cells with water, buffers, or with solutions of the unlabeled compounds. This finding agrees with the much earlier conclusion of Gale and Taylor (7) that GMCD does not cause generalized membrane disruption.

Additional evidence against gross effects on membrane permeability was obtained by measuring the effect of VAL and GMCD on the stability of protoplasts (1, 2). A suspension of protoplasts was diluted into solutions of arabinose, galactose, sucrose, raffinose, glycine, alanine, and valine ranging in concentrations from 0.1 to 0.5 M. Turbidities were measured after 2 hr. The final readings obtained were not materially altered by the addition of either antibiotic. By contrast, tyrocidin at 1 μg/ml caused lysis of protoplasts stabilized by sucrose. By these criteria, then VAL and GMCD selectively increase the permeability of the membrane to cations, but not to other small molecules.

Effect on glycolysis and permeability to H^+. The effect of the antibiotics on glycolysis by *S. faecalis* in water proved to be a function of *p*H (Fig. 2). At *p*H 7.5, addition of VAL had no effect. At *p*H 6, VAL stopped glycolysis, but the inhibition was reversed by addition of KCl or RbCl (1 mM) and of higher concentrations (10 to 100 mM) of NaCl or $MgCl_2$. The effects of GMCD were similar but more drastic: at *p*H 7.5, addition of GMCD

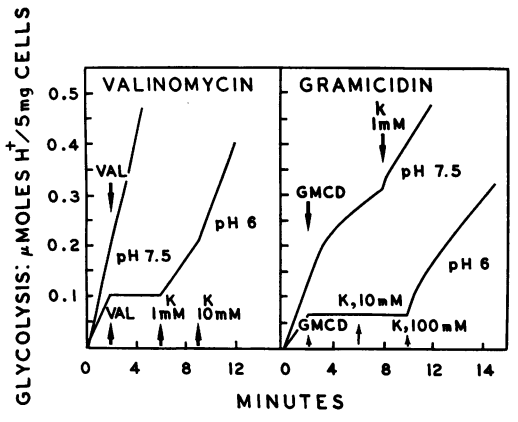

FIG. 2. *Effect of valinomycin and gramicidin on glycolysis. Streptococcus faecalis was harvested during growth on medium KTY. The cells were washed and suspended in water at 1 mg/ml. Glucose (10 μmoles) was added and glycolysis followed on the pH-stat at pH 7.5 or 6. Antibiotics were added to 1 μg/ml at arrows, followed by KCl to final concentrations shown. Abbreviations: valinomycin, VAL; gramicidin, GMCD; KCl, K.*

slowed down the rate of glycolysis, which was restored by cations at 1 mM. At *p*H 6, glycolysis was completely blocked, and high concentrations of KCl or NaCl were required to elicit its resumption.

A clue to an understanding of these phenomena was provided by the observation that addition of VAL or HMCD to a suspension of *S. faecalis* in water was followed by an *increase* in the *p*H; considerable amounts of acid were required to maintain the *p*H at 6. In the experiment illustrated in Fig. 3, the antibiotics were added to a suspension of cells loaded with ^{86}Rb of known specific activity, and a *p*H of 6 was maintained on the *p*H-stat by addition of standardized HCl. At the end of the experiment, the cells were filtered and the filtrates were analyzed for ^{86}Rb. The amount of ^{86}Rb displaced from the cells war stoichiometrically equal to the amount of H^+ consumed, pointing to an uptake of H^+ in exchange for Rb^+.

It would thus appear that VAL and GMCD increase the permeability of the membrane to H^+, which is largely excluded by normal cells (25). H^+ enters the cells in exchange for Rb^+, inhibiting glycolysis by lowering the internal *p*H (25). High concentrations of K^+ or Na^+ displace H^+ by mass action, thus restoring glycolyls. The expected displacement of H^+ by K^+ and Na^+ from cells exposed to VAL or GMCD under the conditions of Fig. 3 was readily observable by use of the *p*H-stat. Displacement of over 90% of the ^{86}Rb by Na^+ did not reduce the rate of glycolysis.

Cation exchanges induced by VAL and GMCD. The cation exchanges induced by VAL and GMCD were examined with respect to their specificity and temperature dependence. Cells were loaded with ^{86}Rb, washed, and resuspended in 10 mM NaCl or RbCl at various temperatures; the *p*H was adjusted to 7.5 (to minimize availability of H^+), VAL was added (1 μg/ml), and samples were taken at intervals. Results are shown in Fig. 4. Displacement of ^{86}Rb by Rb^+ was completed in 2 min at 37 and 20 C and much more slowly at 0 C. Na^+ displaced at 37 and 20 C, although more slowly at 20 C, and had little effect at 0 C. The exchange was approximately stoichiometric. In a particular experiment at 37 C, 400 μmoles of K^+ per g of cells were lost, whereas 250 μmoles of Na^+ were gained (the discrepancy may reflect the exchange between K^+ and H^+ as discussed above). Results similar to those of Fig. 4 were obtained with other cations: K^+ and Cs^+ behaved like Rb^+, whereas Li^+, Mg^{++}, and the cation of tris(hydroxymethyl)-aminomethane (Tris) resembled Na^+.

Exchange of ^{86}Rb by cells treated with VAL at 0 C was inhibited by other cations, particularly polyvalent ones (Fig. 5). Cells were loaded with unlabeled Rb^+, washed, and resuspended in water at 0 C, at *p*H 7.5. VAL was added (1 μg/ml), followed by ^{86}Rb at 0.1 mM. Under these conditions, ^{86}Rb entered the cells by exchange for unlabeled Rb^+; the total Rb^+ content of the cells remained constant. The exchange was inhibited

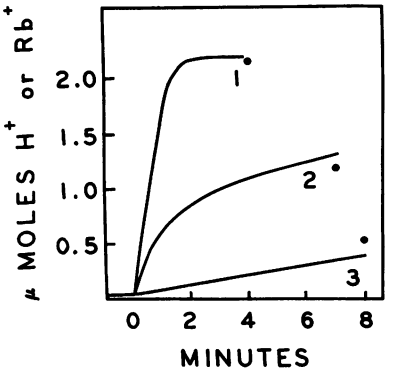

FIG. 3. *Induction of exchange between H^+ and ^{86}Rb by valinomycin and gramicidin. Cells loaded with ^{86}Rb of known specific activity were suspended in water at 0.7 mg/ml. Samples of the suspension were adjusted to pH 6, and additions were made at 0 min: 1) gramicidin, 1 μg/ml; 2) valinomycin, 1 μg/ml; 3) ethyl alcohol only. The pH was maintained at 6.0 on the pH-stat by use of 0.011 M HCl; the lines represent H^+ consumption. At the end, the cells were filtered and the filtrates analyzed for ^{86}Rb. The amount of ^{86}Rb lost from the cells is shown by the circles.*

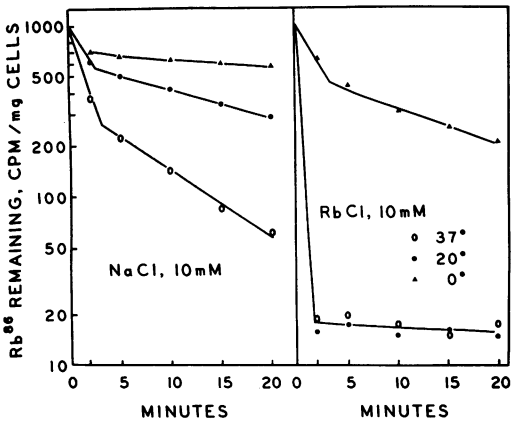

FIG. 4. *Effect of temperature upon the displacement of ^{86}Rb from cells treated with valinomycin by NaCl and RbCl. Cells loaded with ^{86}Rb were washed and resuspended in 10 mM NaCl or RbCl at 37, 20, and 0 C. Valinomycin (1 μg/ml) was added at 0 min. At intervals, 1-ml samples were filtered, and the ^{86}Rb in the cells was determined. The initial ^{86}Rb content was normalized to 1,000 counts per min per mg of cells.*

by NaCl (5 mM) and virtually abolished by Mg^{++} (1 mM), spermine, spermidine, and streptomycin (0.2 mM). Inhibition of exchange between ^{86}Rb and Rb^+ by polyvalent cations was much less pronounced at higher temperatures.

Similar experiments were carried out with GMCD. In this case, displacement of ^{86}Rb by all the cations listed in Table 1 was essentially complete in a few minutes, even at 0 C. The exchanges were again approximately stoichiometric and were but little affected by polyvalent cations (which themselves displaced ^{86}Rb).

Inhibition of energy-dependent K^+ transport. The results presented thus far indicate that VAL and GMCD increase the permeability of the membrane to cations. To determine whether the antibiotics react specifically with the transport system for Rb^+ and K^+ (9*a*), we investigated the effect of the antibiotics upon energy-dependent net uptake of K^+.

Cells grown overnight on medium NaTY are relatively depleted of K^+, but contain large amounts of Na^+ and H^+. In the presence of glucose, such cells take up K^+ with concurrent extrusion of Na^+ and H^+ (9*a*, 26). VAL (1 μg/ml) had little effect upon the uptake of K^+ from water at *p*H 7.5. (Fig. 6). In some experiments, the K^+ that initially accumulated was later displaced again, as Na^+ was added to neutralize the lactic acid formed during glycolysis.

A number of controls were required to buttress the conclusion that VAL does not affect the energy-dependent net uptake of K^+. The net uptake of K^+ and the extrusion of Na^+ were dependent upon glucose both in the absence of VAL and in its presence. Moreover, extrusion of Na^+ (and, of course, uptake of K^+) occurred only when K^+ was provided, both in the absence of VAL and in its presence. It was shown in previous

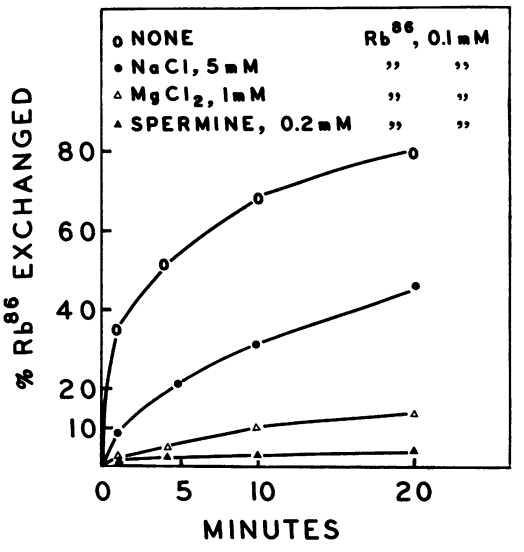

FIG. 5. *Induction of exchange between ^{86}Rb and Rb^+ by valinomycin at 0 C, and its inhibition by other cations. Cells were loaded with Rb^+, washed, and resuspended in water at 0 C. Valinomycin was added (1 μg/ml). After 5 min, additions were made as shown, followed by ^{86}Rb (0.1 mM). Samples were filtered at intervals, washed, and counted. Control suspensions not exposed to valinomycin (omitted for clarity) took up no measurable ^{86}Rb.*

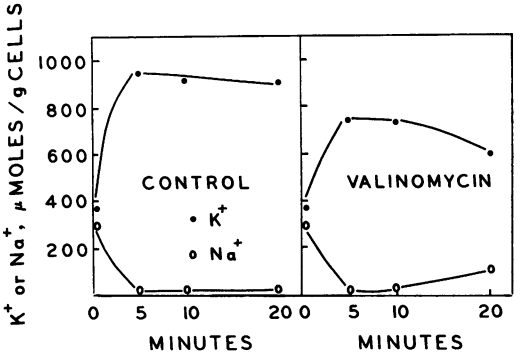

FIG. 6. *Effect of valinomycin on energy-dependent net uptake of K^+. Cells were grown on NaTY, washed, and resuspended in water. Glucose was added and glycolysis initiated at 37 C, pH 7.5. Ethyl alcohol or valinomycin (1 μg/ml) was added, followed 3 min later by 2 mM KCl. Samples were filtered, washed, and analyzed for K^+ and Na^+.*

sections that cells treated with VAL at *p*H 7.5 became permeable to K^+ and Rb^+, whereas Na^+ entered more slowly. Thus the success of the present experiment depends upon careful choice of conditions. The *p*H must be high to minimize availability of H^+, and the Na^+ content of the medium must be kept low to minimize displacement of K^+. At *p*H 6, VAL completely blocked net uptake of K^+ from water.

As expected, parallel experiments with GMCD gave equivocal results. Even in the absence of glucose, 1 mM KCl passively displaced Na^+ (and vice versa). It was therefore impossible to determine whether or not the energy-dependent K^+ transport system continued to function in the presence of GMCD.

Inhibition of energy-dependent transport of ^{32}P-Pi and amino acids. The effect of VAL and GMCD upon the transport of ^{32}P-Pi and amino acids was found to depend upon the cation composition of the medium. Cells of *S. faecalis* were suspended in either sodium maleate or potassium maleate at *p*H 7, 37 C (chloramphenicol, 50 μg/ml, was added to suspensions which later received ^{14}C-amino acids). The suspensions were preincubated with glucose for a few minutes; VAL or GMCD were then added, followed by radioactive substrate. At these concentrations, both Na^+ and K^+ permitted glycolysis to continue.

Results for ^{32}P-Pi and ^{14}C-glutamate are shown in Table 2. Neither VAL nor GMCD inhibited uptake by cells in potassium maleate, but both were inhibitory in sodium maleate. It is probable that inhibition of uptake by the antibiotics is indirect and mediated via their effects on cation permeability.

TABLE 2. *Inhibition of the uptake of ^{32}P-Pi and ^{14}C-glutamate by valinomycin and gramicidin*[a]

Medium	Additions	^{14}C-glutamate[b]	^{32}P-Pi[c]
Potassium maleate.....	Ethyl alcohol only	1,765	3,890
Potassium maleate.....	Valinomycin	1,685	3,495
Potassium maleate.....	Gramicidin	1,785	3,300
Sodium maleate..	Ethyl alcohol only	1,900	2,450
Sodium maleate..	Valinomycin	970	1,000
Sodium maleate..	Gramicidin	150	1,370

[a] *Streptococcus faecalis* was grown on medium KM. The cells were washed and suspended in 0.1 M sodium maleate or potassium maleate at *p*H 7, 37 C. Glucose was added and the cells were incubated for 5 min. To samples of the suspension were added valinomycin (1 μg/ml), gramicidin (1 μg/ml), or ethyl alcohol alone; 3 min later, ^{32}P-Pi (3×10^{-4} M) or ^{14}C-glutamate (1.3×10^{-5} M) were added. The latter suspension also contained 50 μg of chloramphenicol per ml.

[b] Expressed in counts per minute per milligram of cells after 2 min.

[c] Expressed in counts per minute per milligram of cells after 10 min.

DISCUSSION

S. faecalis accumulates large amounts of K^+ and Rb^+ during growth (*9a*), apparently in association with various phosphorylated compounds. These cations are tenaciously retained and exchange but slowly with external cations in the absence of a source of metabolic energy (*9a*). The permeability barrier to cations presumably resides in the cytoplasmic membrane, since cations are lost when the cells are treated with agents known to disrupt the membrane such as organic solvents, detergents, or tyrocidin (4, 7, 8). The cation barrier is exceedingly sensitive to the antibiotics GMCD and VAL. These substances in concentrations of the order of 10^{-8} to 10^{-7} M markedly increase the rate of exchange of cations across the membrane, but (unlike the nonspecific agents enumerated above) have no apparent effect on the permeability to other small molecules. It must be stressed here that cation exchanges induced by GMCD and VAL do not require an exogenous substrate and take place even at 0 C. *S. faecalis* apparently lacks internal energy reserves, since transport of ^{32}Pi, ^{14}C-glutamate, ^{86}Rb, and K^+ by normal cells is strictly dependent upon an exogenous substrate (9, *9a*, 26); thus, the cation exchanges induced by the antibiotics must be metabolically passive.

Of the two antibiotics, GMCD has the more drastic effects. Even at 0 C, GMCD-treated cells rapidly exchange internal Rb^+ or K^+ for external cations ranging from H^+ to Mg^{++}, and more slowly for bulky cations like Tris and spermine. These results agree well with the findings of Chappel and Crofts (3) in mitochondria.

The effects of VAL are more subtle. In the presence of VAL, ^{86}Rb exchanges rapidly for H^+, K^+, Rb^+, and Cs^+, more slowly for Na^+, Li^+, Mg^{++}, and the cation of Tris. This suggests an inverse relationship between the rate of entry of the cation and its hydrated ionic radius (23). The rate of exchange also depends strongly upon the temperature. At 0 C, cation exchanges are strongly inhibited by polyvalent cations which are believed (8, 24) to react with the membrane. The kinetics of exchange are complex and do not fit a

single exponential (Fig. 4, 5); detailed kinetic analysis was not carried out.

Previous studies (9*a*, 26) on cation uptake by *S. faecalis* have furnished evidence for the existence of specific transport systems which mediate selective, energy-dependent uptake of K^+ and Rb^+. From the results presented here (Fig. 6), it appears that VAL neither stimulates nor inhibits this K^+ pump, but rather affects cation permeability of the membrane in general. This finding differs from that of Pressman and coworkers (10, 11, 20) who concluded that VAL stimulates the K^+ pump of mitochondria. We were unable to determine whether the K^+ pump continues to function in the presence of GMCD.

The increased permeability of the membrane to cations is a sufficient explanation for the inhibition of growth by VAL and GMCD and its reversal by K^+. The action of GMCD and VAL is thus reminiscent of that of certain polyene antibiotics on fungal cells (4). A high internal concentration of K^+ is required for growth of *S. faecalis* (9*a*) and of *Escherichia coli* (13). In *E. coli*, the process most sensitive to K^+ deprivation is protein synthesis (13). The situation *S. faecalis* may be more complex; although glycolysis does not require K^+ (1, 2), a variety of transport processes appears to be impaired in cells deficient of K^+. The present findings confirm the involvement of K^+ in the uptake of amino acids (5, 16), sugars (2), and phosphate (9), and provide a tool which may help clarify the role of K^+ in these diverse processes.

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